Effect of Pretreatments on Controlling Enzymatic Browning Before Drying of Eggplant Slices

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ABSTRACT

The present study was carried out to investigate polyphenoloxidase inhibition in eggplant slices before drying. Pretreatments were carried out single or combined from immersion in ascorbic acid, citric acid or sodium chloride solutions at different concentrations 0.5, 1.0, 1.5, 2.0% and /or steaming for 2,4,6,8 min. Polyphenoloxidase activity of fresh eggplant slices was 0.65 unit /g/min. Sever inhibition was detected with increasing chemical inhibitors concentration or steam regardless of chemical inhibitors types .The inhibitory effect of steam pretreatments or various chemicals inhibitors on eggplant slices was found to decrease in the following order steam treatment > ascorbic acid > sodium chloride > citric acid at the same concentration level. Combination of chemical inhibitors and steam treatment (Ascorbic acid, citric acid, sodium chloride at levels 0.5% and steam treatment for 2min) as hurdle technology resulted most effective inhibition on enzymatic browning. Dried eggplant slices with aforementioned combined pretreatment at low concentration of chemical inhibitors and steam were less three times of enzymatic browning index than control treatment (without pretreatment). Also, inhibition of polyphenoloxidase of eggplant slices before drying were higher maintained bioactive compounds especially phenolic compounds, ascorbic acid and antioxidant activity than control. .

Keywords: polyphenoloxidase, eggplant, inhibition, drying.

INTRODUCTION

Eggplant (*Solunum melongena L*. is a plant of the family *Solanaceae*, is one of the important vegetables grown and consumed in Egypt and other tropical countries. The annual production of eggplant in Egypt was estimated to be 1166430 tons produced from a total area of 170.190.48 Feddans (FAO, 2013). Eggplants which are low in calories, have high water content, and provides relatively high levels of calcium, phosphorus, potassium, sodium, fiber, folic acid, and vitamins B and C (Kashyap *et al.,* 2003). Also, eggplant fruit contains ascorbic acid and phenolics, both of which are powerful antioxidants (Vinson *et al.,* 1998). Eggplant has a very limited shelf life for freshness and deteriorate during long term storage and associated with pulp browning caused by the oxidation of phenolic compounds. The level of phenolics has been shown to correlate with browning in different eggplant varieties (Prohens *et al.,* 2007). Color determine acceptance of food by the consumer being the first factor that determines the acceptance or rejection of food .Thus ,the preservation of the original color of food during processing is a fundamental to the acceptability of product derived from fruits or vegetables (Almida and Nogueira,1995). In the oxidative degradation of the phenolic compounds there are two relevant enzymes involved whose activity can lead to the production of brown polymers.These enzymes are the polyphenoloxidase (PPO, EC 1.14.18.1) and peroxidase (Tomas-Barberán and Espin, 2001). The enzymatic activity of vegetable tissues is one of the principal causes of food nutritional and organoleptical impairment. Polyphenoloxidase catalyzes the initial step in the polymerization of phenolics to produce quinones, which undergo further polymerization to insoluble dark brown polymers known as melanins. The browning in vegetables is controlled by the levels of phenols, activity of PPO and the presence of oxygen (Spanos and Wrolstad, 1992).

o-quinones are very unstable and rapidly react with amino acids or proteins, generating brown pigments by polymerization (Garcia-Carmona *et al.,* 1988). Also, Lozano (2006) reported that PPO activity may be minimize by reducing agents, heat inactivation, lowering the pH of the product, and the presence of enzyme inhibitors. The most commonly used inhibition method in food industry has been the addition of sulfur compounds. They are used to inhibit the browning of fruits and vegetables, as well

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as whitening compounds and inhibiting the growth of microorganisms. Although these compounds, very effective in the inhibition of both enzymatic and non-enzymatic browning, may cause allergic reactions and have a negative effect on taste (Bieganska and Czapski,2007). Dehydration constitutes an alternative to provide higher stability eggplant products, which may be shipped to external markets or used along the whole year (Akpinar and Bicer, 2005). Dried eggplant could be used as a new ingredient in foodstuffs, like soups and sauces Greek moussaka and Baba Ganoush. Also, dried eggplant is traditionally consumed in different kinds of meals. Samappito & Trachoo (2011) reported that instant fiber powder from some vegetables such as eggplant was found successful supplemented with probiotic bacteria in dairy products.

The aim of this work was to evaluate the browning inhibitors of eggplant before drying to produce high quality dried eggplant powder.

MATERIALS AND METHODS

Materials: Fresh eggplant *(Solunum melongena L.*) purple colored were purchased from the local market, Giza, Egypt in April 2012..

Pretreatments for controlling browning

Eggplants were washed in running water, drained, peeled, the flesh cut into 1 cm-thick slices .The slices of eggplant were dipped into solutions at different concentrations of ascorbic acid, citric acid and sodium chloride at ratio slices eggplant: solution,1:2 (w/v) for 15 min. or blanched with steam at different times. Also, the competitive inhibition of the lowest concentrations of ascorbic acid, citric acid and sodium chloride in solutions or steam were combined to use inhibiting PPO activity in Table (1).

Drying of eggplant slices

The highest pretreatment of polyphenoloxidase inhibition of eggplant slices and fresh as control were dried at 60ºC in oven under vacuum until constant weight then ground by petern laboratory mill and sieved (0.125 mesh) then, packed in laminate sacs PPA packing material (polyethylene, polyester and aluminum foils).

Analytical methods

Moisture content, pH value , total acidity, fiber, ash, ascorbic acid , reducing ,non-reducing ,total sugars, protein and fat contents were determined according to A.O.A.C (2005). Browning index of dried and fresh samples was determined as described by Ranganna (1977). The total phenolic content as catechol (mg/100g) was determined as described by Waterman and Mole (1994). Antioxidant activity of samples extracts was studied through the evaluation of the free radical-scavenging effect on the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical. The results were expressed as percentage of inhibition

Table 1: Pretreatments of eggplant slices

Treatments	Concentration %
Control	-
(T_1) Ascorbic acid	0.5-1.0- 1.5 - 2.0
(T_2) Citric acid	0.5-1.0- 1.5 - 2.0
(T_3) Sodium chloride	0.5-1.0- 1.5 - 2.0
(T_4) Steam	2- 4-6 - 8 min
($T_{5)}$ Ascorbic acid + Citric acid	0.5+ 0.5
(T6) Ascorbic acid + Sodium chloride	0.5+ 0.5
(T_7) Ascorbic acid + Steam	0.5+ 2.0 min
($T_{8)}$ Citric acid+ Sodium chloride	0.5+ 0.5
($T_{9)}$ Citric acid+ Steam	0.5+ 2.0 min
(T_{10})Sodium chloride+ Steam	0.5+ 2.0min
(T_{11})Ascorbic acid+ Citric acid + Steam	0.5+0.5+2.0 min
(T_{12})Ascorbic acid+ Sodium chloride + Steam	0.5+0.5+2.0 min
(T_{13})Ascorbic acid+ Citric acid + Sodium chloride	0.5+0.5+0.5
(T_{14})Ascorbic acid+ Citric acid + Sodium chloride + Steam	0.5+0.5+0.5+2.0 min

of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to Alothman *et al*. (2009) using the following equation:-

$$\% \text{ inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where: Abs control is the absorbance of DPPH solution without extracts, Abs sample is the absorbance of DPPH with solution extracts.

Determination of polyphenoloxidase (PPO) activity

Polyphenoloxidase activity of fresh and after pretreatments of eggplant slices was determined according to the method of Shatta & EI-Shamei (1999) as follows:

Preparation of enzyme extract

Sample (10g) was homogenized using a Homogenizer (type: T25B Jkika Labortechnik, Germany) under cooling for 3 min at 1 min intervals for mixing with 50 ml 0.2 M sodium phosphate buffer (pH 6.5). Homogenates were centrifuged for 30 min at 18000 g at 4°C. The enzyme extract was ready for assaying.

Assay of enzyme activity

The PPO activity was determined at 25°C by measuring the initial rate of increase in absorbance at 420 nm. Activity was assayed in a 3 ml reaction mixture, consisting of 0.05 M catechol in 0.05 M sodium phosphate buffer (2.75 ml, pH 6.5) and aliquots of enzyme extract (0.25 ml). The enzyme unit was defined as the change in absorbance in unit / min / g sample of eggplant slices.

Statistical analysis

The obtained data were statistically analyzed using ANOVA procedure of the SPSS statistical package at confidence level of 0.05 (SPSS, 1990).

RESULTS AND DISCUSSION

Physical and chemical properties of fresh eggplant

Physical and chemical properties of eggplant (*Solunum melongena* L.) fruits are shown in Table (2). The results indicate that the peel, pulp and TSS were 26.9, 73.1 and 5.5%, respectively. The data show that total acidity, total sugars, fat, protein, ash and crude fiber were 3.18, 53.85, 6.71, 8.28, 3.59 and 22.46% of fresh eggplant on dry weight basis, respectively. These results are in agreement with those reported by Nezam El-Din & Omar (1991) and Nisha *et al*. (2009). Polyphenols represent a large group of phytochemicals that are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables. Because of their chemical structure, plant polyphenols can scavenge free radicals and inactive other pro-oxidants, and also interact with a number of biological relevance (Nisha *et al*., 2009).

Table 2: Physico-chemical properties of fresh flesh eggplant

Properties	Values
Peel %	26.90
Pulp %	73.10
TSS %	5.50 ± 0.208
Moisture %	91.36 ± 0.11
pH value	5.36 ± 0.08
Total acidity* % (as citric acid)	3.18 ± 0.07
Total sugars* %	53.85 ± 0.118
Reducing sugars *%	24.23 ± 0.142
Non Reducing sugars* %	29.62 ± 0.072
Fat* %	6.71 ± 0.115
Protein* %	8.28 ± 0.078
Ash* %	3.59 ± 0.056
Crude fiber* %	22.46 ± 0.092
Total phenolic compounds (mg/100g)	77.28 ± 0.061
Ascorbic acid (mg/100g)	330.55 ± 0.57
Antioxidant activity %	81.46 ± 0.12
Browning index (at 420nm)	0.167 ± 0.002

*On dry weight basis

Table (2) illustrates that the total phenolic content, ascorbic acid and antioxidant activity in flesh eggplant are 77.28, 330.55 mg /100g fresh weight and 81.46%, respectively. The results indicate that eggplant extract is a good source of phenolic and ascorbic acid compounds and has more benefits to human health. These results are in harmony with those reported by Hung & Duy (2012).

Effect of pretreatments on PPO activity of eggplant slices

The activity (unit/g/min) and % inhibition of PPO in the eggplants slices of different treatments are presented in Table (3). Acids prevent browning are widely used and fall within the group of acids naturally present in vegetable tissues such as citric, malic, phosphoric and ascorbic acids. In general, the effect of inhibition is due to the low pH.

Table 3: Activity of PPO after pretreatments of eggplant slices

Treatments	Activity (unit/g/min)	Inhibition (%)
Control	0.650±0.004q	0.00 [a]
(T_1) Ascorbic acid		
0.5%	0.371±0.003n	42.92±0.02[d]
1.0%	0.204±0.004 j	68.61±0.015[k]
1.5%	0.131±0.002g	79.84±0.15[o]
2.0%	0.101 ±003e	84.46±0.15[q]
(T_2) Citric acid		
0.5%	0.514±0.005p	20.92±0.075[b]
1.0%	0.357±0.003n	45.07±0.135[e]
1.5%	0.283±0.006[m]	56.46±017[f]
2.0%	0.207±0.005ij	68.15±0.167[j]
(T_3)Sodium chloride		
0.5%	0.40 ±0.005o	38.46±0.443[c]
1.0%	0.255±0.006l	60.76±0.123[g]
1.5%	0.232±0.009k	64.30±0.38[h]
2.0%	0.185±0.111h	71.54±0.229[m]
(T_4) Steam		
2 min	0.210±0.02j	67.69±0.184[i]
4 min	0.110±0.005[ef]	83.07±0.171[p]
6 min	0.065±0.012[cd]	90.00±0.15[t]
8 min	0.022±0.004[ab]	96.61±0.287[x]
(T_5) Ascorbic acid + Citric acid	0.191±0.006[hi]	70.61±0.34[l]
(T_6) Ascorbic acid + Sodium chloride	0.12±0.005 [fg]	75.57±0.265[n]
(T_7) Ascorbic acid + Steam	0.063±0.013[cd]	88.75±0183[s]
(T_8) Citric acid+ Sodium chloride	0.253±0.009l	61.07±0.151[g]
(T_9) Citric acid+ Steam	0.130±0.032g	80.00±0.185[o]
(T_{10})Sodium chloride+ Steam	0.08 ±0.006 d	87.68±0.282[r]
(T_{11})Ascorbic acid+ Citric acid + Steam	0.048±0.003c	91.42±0.215[v]
(T_{12})Ascorbic acid+ Sodium chloride + Steam	0.031±0.013b	95.23±0.187[b]
(T_{13})Ascorbic acid+ Citric acid + Sodium chloride	0.062±0.003[cd]	90.46±0.123[cd]
(T_{14})Ascorbic acid+ Citric acid + Sodium chloride + Steam	0.008±0.154[a]	98.76±0.21[a]

Means within a column showing the same letters are not significantly different (P≥ 0.05).

The inhibitory effect of ascorbic acid is due mainly to the reduction of the orthoquinones, generated by the action of PPO, to the original orthophenolics. Ascorbic acid delayed up to the point at which all of the ascorbic acid is oxidised (Almida & Nogueira, 1995). When the concentrations of ascorbic and citric acids or sodium chloride in the solutions increased, the PPO accompanied by markable decrease in activity. The results in Table (3) and Figs. (1 & 2) show that inhibition of polyphenoloxidase were higher by ascorbic acid as compared with citric acid at the same level .It could be attr to that ascorbic acid acts also, as scavenger by removing molecular oxygen from the reactions catalysed by PPO. Also, it has a chelating effect on cupper present in the prosthetic group of the enzyme, which is consistent with acidity differences as reported by Zhu *et al*. (2014).

The results in Table (3) indicated that low percentage of citric acid in immersion solution led to a slight decrease of PPO activity. Peng & Jiang (2006) reported that citric acid at a concentration lower than 0.02 M (3.84 g/ L) stimulated PPO activity of fresh-cut Chinese water chestnut, but at 0.1 M (19.2 g/ L) or higher markedly inhibited the activity.

The same table and Fig. (3) illustrated that sodium chloride was more effect inhibition of eggplant PPO than those treated with citric acid at the same level. Lu *et al*., (2007) reported that apple slices treated in sodium chloride solution had a significantly decrease browning than those treated in citric acid or water control at 4 h ($P < 0.01$).Sodium chloride is well –Known inhibitors of PPO, the inhibitory effect of sodium chloride is attributed to the chloride ion. It was noticed that inhibition of PPO by sodium chloride was higher than that of citric acid but it had effect on product taste (Vlasta & Subaric, 1998).

Eggplant slices were blanched for 2, 4, 6 and 8 min by steam, (Fig. 4) for fresh eggplant slices to inhibit PPO,the increasing time of blanching was accompanied rapidly by decreasing in polyphenoloxidase. High activation energy reflects a greater sensitivity of PPO to temperature change and the extent of PPO denaturation increased with treatment time thus, residual activity was only about 7% after 5 min at 85 °C and 1.2% after 5 min at 90°C (Chutintrasri & Noomhorm, 2006). Ali *et al.*, (2011) reported that blanching as a pretreatment is a short-time heat-

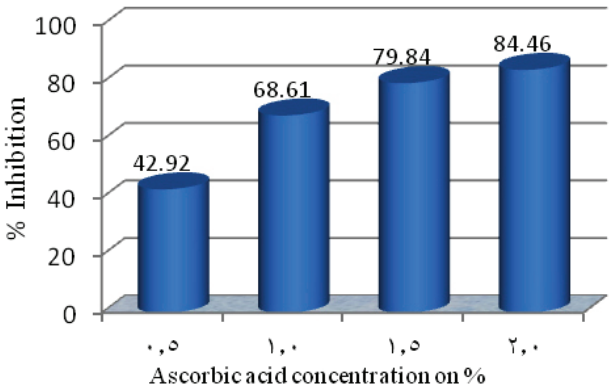

Fig. 1: Effect of various ascorbic acid concentration on % inhibition of PPO

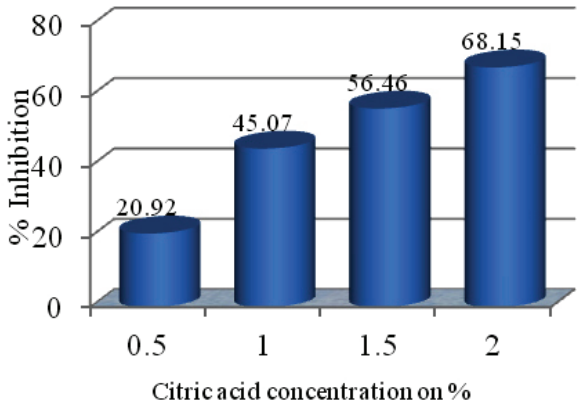

Fig. 2: Effect of various cetric acid concentration on % inhibition of PPO

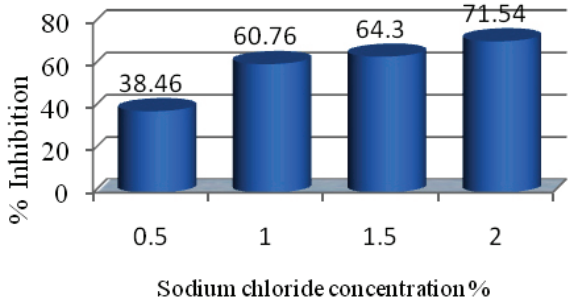

Fig. 3: Effect of various sodium chloride acid concentration on % inhibition of PPO

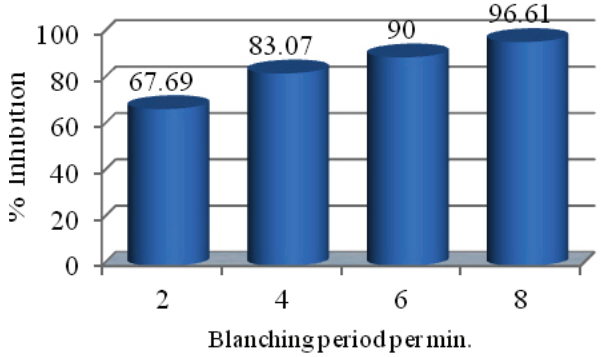

Fig. 4: Effect of various blanching periods on % inhibition of PPO

treatment generally applied to vegetables primarily to inactivate natural enzymes. Use of this agent has several disadvantages such as negative effects on the texture, color alterations, flavor damage and vitamin and nutritional losses.

Polyphenoloxidase (PPO) is most active at pH 7. It means that acidifiers which decrease pH below inhibit the enzymatic activity of PPO. It could be affecting adversely of sensory properties. PPO can also inhibited by heat treatment which causes the degradation of food components and impairs the taste and low quality of the texture products. Thus, combination pretreatments to inhibit polyphenoloxidase were used in factories as ahurdle technology. The most effective of pretreatment to inhibit polyphenoloxidase were noticeable in presence steam followed by ascorbic acid, sodium chloride, and then citric acid. Results in the same Table (3) and Fig. (5) indicated that the best treatment (T_{14}) inhibited 98.76% of polyphenoloxidase activity. Thus pretreatment was chosen for subsequent drying by air at 60C.

Effect of drying on physical and chemical properties of eggplant powder.

Physical and chemical properties after drying of eggplant powder then, powdered recorded in Table (4). Moisture content of eggplant powder of control (without pretreatment) and treatment (T_{14}) were 7.24 and 8.45%, respectively. The low percentages of moisture content of eggplant powder illustrate lower sugars and higher of crude fiber in fresh eggplant. Acidity was slight decreased after drying for all treatments nevertheless; acidity of control was higher decreased than that treatment (T_{14}). This difference might be due to oxidation of some acids during drying and the reaction between sugars and organic acids.

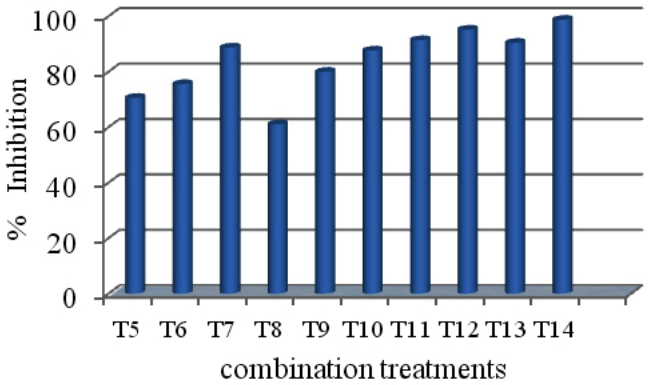

Fig. 4: Effect of combination treatments on % inhibition of PPO

Table 4: physical and chemical properties of eggplant powder

Properties	Control±SE	$T_{(14)}$±SE
Moisture %	7.24 ± 0.04	8.45 ±0.13
pH value	5.40 ±0.015	5.30 ±0.25
Total acidity* % (as citric acid)	2.88 ±0.05	3.01 ±0.09
Total sugars* %	52.65 ±0.21	52.25 ±0.09
Reducing sugars* %	29.23 ±0.157	32.40 ±0.455
Non Reducing sugars* %	23.42 ±0.37	19.85 ±0.45
Fat* %	6.54 ±0.08	6.56 ±0.03
Protein* %	7..91 ±0.10	7.85 ±0.11
Ash* %	3.52 ±0.10	3.65 ±0.046
Crude fiber* %	22.34 ±0.097	22.23 ±0.35
Total phenolic compounds mg/100g	54.62 ±0.09	69.18 ±0.10
Ascorbic acid mg/100g	182.71 ±0.12	264.50 ±0.18
Browning index (at420nm)	1.325 ±0.006	0.427 ±0.003
Antioxidant activity %	48.76 ±0.06	59.63 ±0.24

*On dry weight basis

Total sugars decreased by 2.23-2.97% of the initial value for samples due to the effect of temperature on properties of eggplant slices. This observation could be due to the contributions of reducing sugars to Millard reactions. It could be noticed that non-reducing sugars decreased along the drying processes with the immersion in acid solution. This increment in non-reducing sugars may be due to the conversion of non-reducing sugars into reducing form.

Results in Table (4) illustrated that fat and protein content of eggplant powder were slightly decrease after drying regardless of with and without pretreatment used. Also, in the same table ash content of treatment (T_{14}) was higher than that control treatment. It may be due to transfer of solute (sodium chloride) from solution to product (Raoult-Wack *et al* ,1994).

Phenolic compounds are generally sensitive to adverse environmental conditions, including unfavourable temperatures, light, pH, moisture, and oxygen, and are therefore susceptible to degradative reactions during product processing. Total phenloic compounds of treatment (T_{14}) were contained higher than control ones. Ascorbic acid in control treatment was lower than treatment (T_{14}). This lower quantity of ascorbic acid of control treatment may be attributed to oxidation reaction accelerated by the heating process and dependent on phenolic compounds and PPO levels (Chow *et al*., 2011). Also, it could be pretreated with ascorbic acid, sodium chloride, citric acid and heat treatment before drying which it inhibited PPO and scavenging of materials which may reduce the phytochemical compounds.

Browning index (at 420 nm) was increased about 7 times of initial value in control treatment while treatment (T_{14}) was increased by about 1.5 times of initial value .The global improvement of end product quality due to the pretreatment has been also confirmed through the experimental analysis, which has been shown both the reduction of browning phenomena and the preservation of chemical compounds.

In conclusion the most effective inhibitory treatment for PPO activity of eggplant slices before drying were combined pretreatment contained of ascorbic acid ,sodium chloride and citric acid (0.5+0.5+0.5%), at ratio slices eggplant: solution (1:2, w/v) for 30 min, then treated with steam for 2min.

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تأثير بعض المعاملات الأولية للتحكم فى التلون الإنزيمي قبل تجفيف شرائح الباذنجان

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أجريت هذه الدراسة لبحث تثبيط إنزيم البولي فينول أوكسيديز في شرائح الباذنجان قبل التجفيف. وقد أجريت المعاملات الأولية منفردة أومجتمعة بالغمر في حمض الأسكوربيك، حمض الستريك أوكلوريد الصوديوم بتركيزات مختلفة ٠,٥، ١,٠، ١,٥ ، ٢,٠ ٪ أو بالبخار لمدة ٢،٤،٦،٨ دقيقة . كان النشاط الإنزيمى لإنزيم البولى فينول أوكسيديز لشرائح الباذنجان الطازج ٠,٦٥ وحدة / جم / دقيقة. أوضحت النتائج أن زيادة تثبيط الانزيم متلازماً مع زيادة تركيز المثبطات الكيميائية أو البخار بغض النظر عن نوع المثبط الكيميائى. وجد أن درجة نشاط انزيم البولى فينول أكسيديز ينخفض حسب الترتيب التالي المعاملة بالبخار > حمض الاسكوربيك > كلوريد الصوديوم > حامض الستريك عند نفس مستوى التركيز. كما أوضحت النتائج أن الجمع بين المثبطات الكيميائية والمعاملة بالبخار(تكنولوجيا العقبات) أعلى تثبيطياً لأنزيم البولى فينول أوكسيديز عند تركيز٠,٥ ٪ حمض الأسكوربيك و حمض الستريك وكلوريد الصوديوم والمعاملة بالبخار لمدة ٢ دقيقة. وجد أن التلون الإنزيمى لشرائح الباذنجان المجففة والمعاملة بأقل تركيز من المثبطات الكيميائية والبخار أقل بثلاث مرات من العينة المقارنة ، أيضا حافظ تثبيط البولي فينول أوكسيديز لشرائح الباذنجان قبل التجفيف على المركبات الحيوية وبخاصة المركبات الفينولية وحمض الأسكوربيك ودرجة نشاط مضادات الأكسدة للباذنجان المجفف مقارنة بالعينة المقارنة.